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Supplemental Information

Sam50 Regulates PINK1-Parkin-Mediated Mitophagy by Controlling PINK1 Stability and Mitochondrial Morphology

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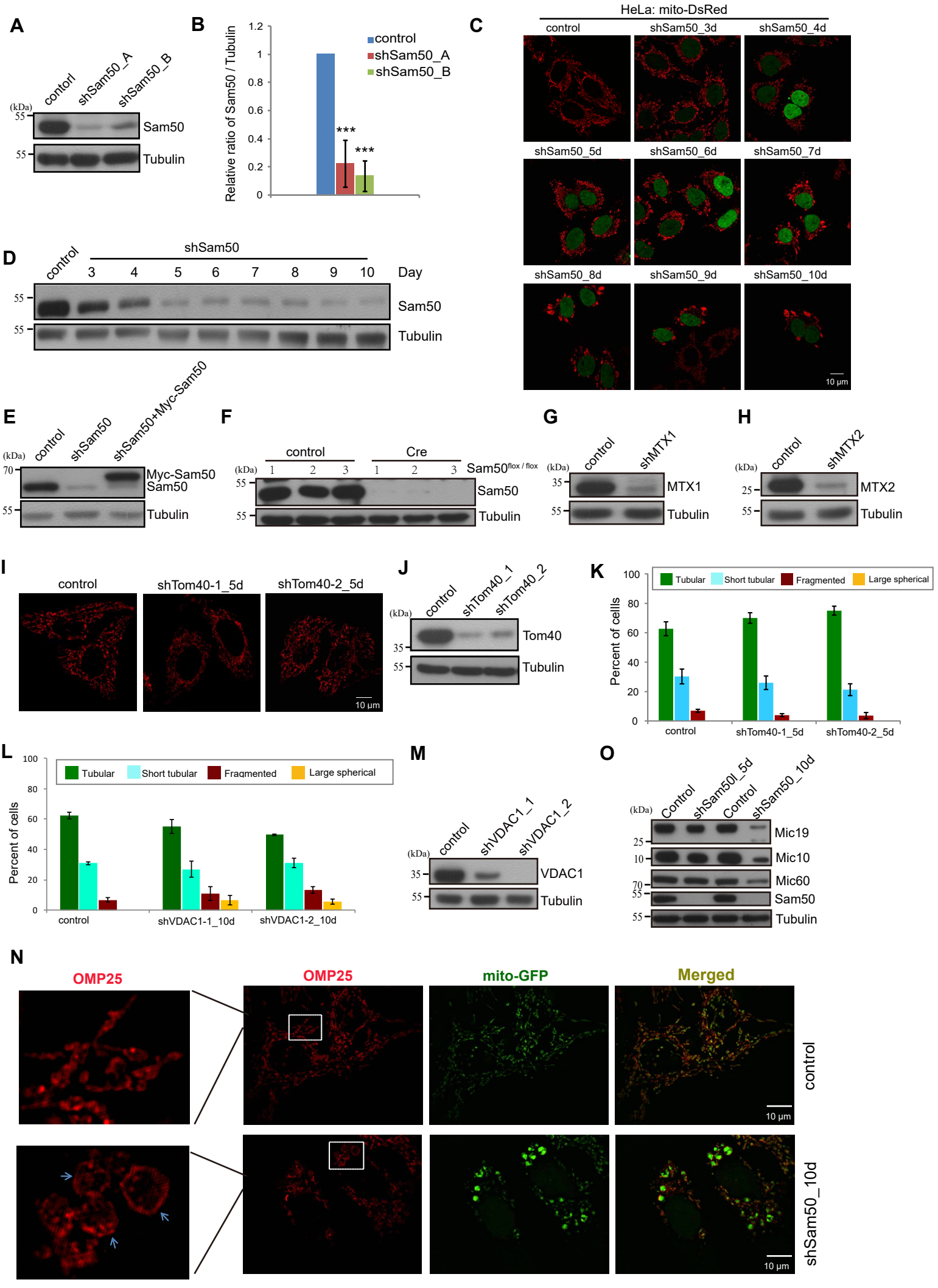

A
control
shSam50_A
shSam50_B
(kDa)
55
Sam50
55
Tubulin
B
Relative ratio of Sam50 / Tubulin
1.2
1
0.8
0.6
0.4
0.2
0
control
shSam50_A
shSam50_B
***
***
C
HeLa: mito-DsRed
control
shSam50_3d
shSam50_4d
shSam50_5d
shSam50_6d
shSam50_7d
shSam50_8d
shSam50_9d
shSam50_10d
10 μm
D
control
shSam50
3
4
5
6
7
8
9
10
Day
55
Sam50
55
Tubulin
E
control
shSam50
shSam50+Myc-Sam50
(kDa)
70
Myc-Sam50
Sam50
55
Tubulin
F
control
Cre
(kDa)
1
2
3
1
2
3
Sam50flox / flox
55
Sam50
55
Tubulin
G
control
shMTX1
(kDa)
35
MTX1
55
Tubulin
H
control
shMTX2
(kDa)
25
MTX2
55
Tubulin
I
control
shTom40-1_5d
shTom40-2_5d
10 μm
J
control
shTom40_1
shTom40_2
(kDa)
35
Tom40
55
Tubulin
K
Tubular
Short tubular
Fragmented
Large spherical
Percent of cells
100
80
60
40
20
0
control
shTom40-1_5d
shTom40-2_5d
L
Tubular
Short tubular
Fragmented
Large spherical
Percent of cells
100
80
60
40
20
0
control
shVDAC1-1_10d
shVDAC1-2_10d
M
control
shVDAC1_1
shVDAC1_2
(kDa)
35
VDAC1
55
Tubulin
O
Control
shSam50l_5d
Control
shSam50_10d
(kDa)
25
Mic19
10
Mic10
70
Mic60
55
Sam50
55
Tubulin
N
OMP25
OMP25
mito-GFP
Merged
control
shSam50_10d
10 μm
10 μm

Supplemental Data

Figure S1 (Related to Figure 1): The effect of VDAC1 or Tom40 on mitochondrial morphology.

(A-B) Control and shSam50 HeLa cells were subjected to immunoblot for Sam50 and Tubulin (A). Quantification of the relative Sam50 levels by densitometry analysis using ImageJ software (B). Error bars indicate the mean ±SD of three independent experiments, ***P < 0.0001.

(C-D) The mitochondrial morphology of shSam50 HeLa cells expressing mito-DsRed at the indicated time was visualized by confocal microscope (C). The level of Sam50 was measured by immunoblotting (D).

(E) HeLa cells expressing Myc-Sam50 (mouse Sam50) were infected with control or shSam50 lentiviral particles, the cell lysates were subjected to immunoblot using the indicated antibodies.

(F) $Sam50^{flox/flox}$ MEFs were infected with control or Cre retroviral particles and cultured for 10 days. The whole cell lysates were evaluated by immunoblot analysis using Sam50 antibody.

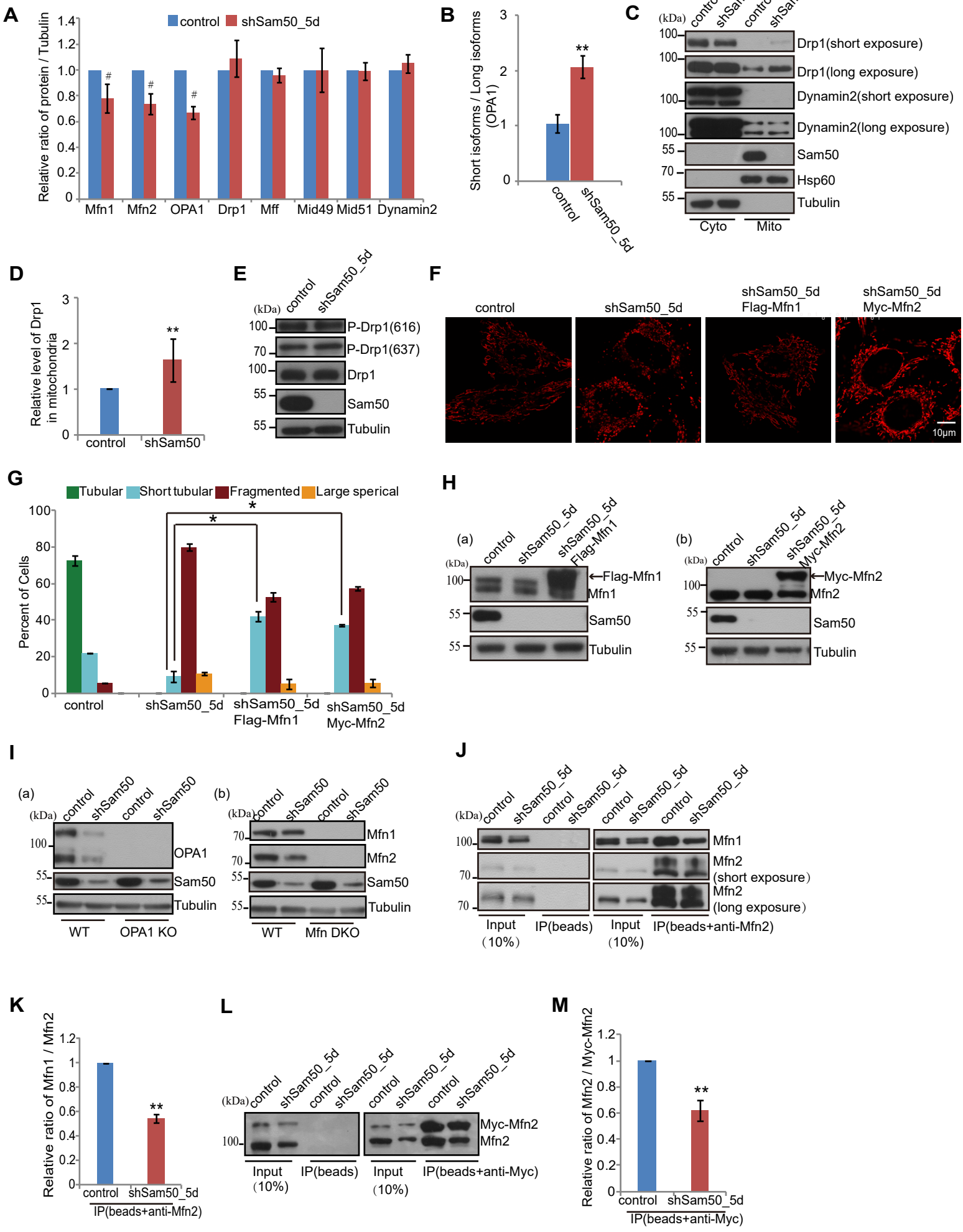
(G-H) The level of Metaxin1 and Metaxin2 were analyzed by immunoblot analysis using the indicated antibodies.

(I-K) Mitochondrial morphology in control or shTom40 HeLa cells expressing mito-DsRed were visualized by confocal microscope (I), then was counted according to the criteria detailed in “Experimental Procedures” (K). Data are expressed as means ± SD. The effect of Tom40 knockdown was analyzed by immunoblot using anti-Tom40 and anti-Tubulin antibodies (J).

(L-M) Mitochondrial morphology of control and shVDAC1 HeLa cells was counted according to the criteria detailed in “Experimental Procedures”. Error bars represent means ± SD of three independent experiments (L). The level of VDAC1 was analyzed by immunoblot using anti-VDAC1 antibody (M).

(N) Control or shSam50 HeLa cells were cultured continuously for 10 days, mitochondria in cells expressing mito-GFP (green) were immunostained for OMP25 (red, anti-OMP25), the images were acquired with a super-resolution structured illumination microscopy. The arrow indicates the large spherical mitochondrion.

(O) The protein levels of MICOS complex components in control or shSam50 (5 or 10days) HeLa cells were analyzed by immunoblotting with the indicated antibodies.


A
control
shSam50_5d
Relative ratio of protein / Tubulin
Mfn1
Mfn2
OPA1
Drp1
Mff
Mid49
Mid51
Dynamin2
B
Short isoforms / Long isoforms (OPA1)
control
shSam50_5d
C
(kDa)
Drp1(short exposure)
Drp1(long exposure)
Dynamin2(short exposure)
Dynamin2(long exposure)
Sam50
Hsp60
Tubulin
Cyto
Mito
D
Relative level of Drp1 in mitochondria
shSam50
E
P-Drp1(616)
P-Drp1(637)
Drp1
Sam50
Tubulin
F
shSam50_5d Flag-Mfn1
shSam50_5d Myc-Mfn2
10μm
G
Tubular
Short tubular
Fragmented
Large sperical
Percent of Cells
shSam50_5d Flag-Mfn1
shSam50_5d Myc-Mfn2
H
(a)
(b)
Flag-Mfn1
Mfn1
Myc-Mfn2
Mfn2
I
OPA1
WT
OPA1 KO
Mfn DKO
J
Mfn2 (short exposure)
Mfn2 (long exposure)
Input (10%)
IP(beads)
IP(beads+anti-Mfn2)
K
Relative ratio of Mfn1 / Mfn2
IP(beads+anti-Mfn2)
L
IP(beads+anti-Myc)
M
Relative ratio of Mfn2 / Myc-Mfn2
IP(beads+anti-Myc)

Supplemental Data

Figure S2 (Related to Figure 2): The role of mitochondrial fusion or fission factors in shSam50 induced abnormal mitochondrial morphology.

(A) The protein levels of fusion and fission factors in control or shSam50 (5days) HeLa cells were further evaluated by densitometry analysis using ImageJ software. Error bars indicate the mean ±SD of three independent experiments, $^{\#}P<0.05$.

(B) The ratio of Short isoforms / Long isoforms of OPA1 was quantified in control or shSam50 (5 days) HeLa cells. Error bars indicate the mean ±SD of three independent experiments, **P<0.001.

(C-D) Control or shSam50 (5 days) HeLa cells were fractionated into cytosolic and mitochondrial fractions, then evaluated by immunoblot analysis as indicated. The level of Drp1 in mitochondria was then quantified (D).

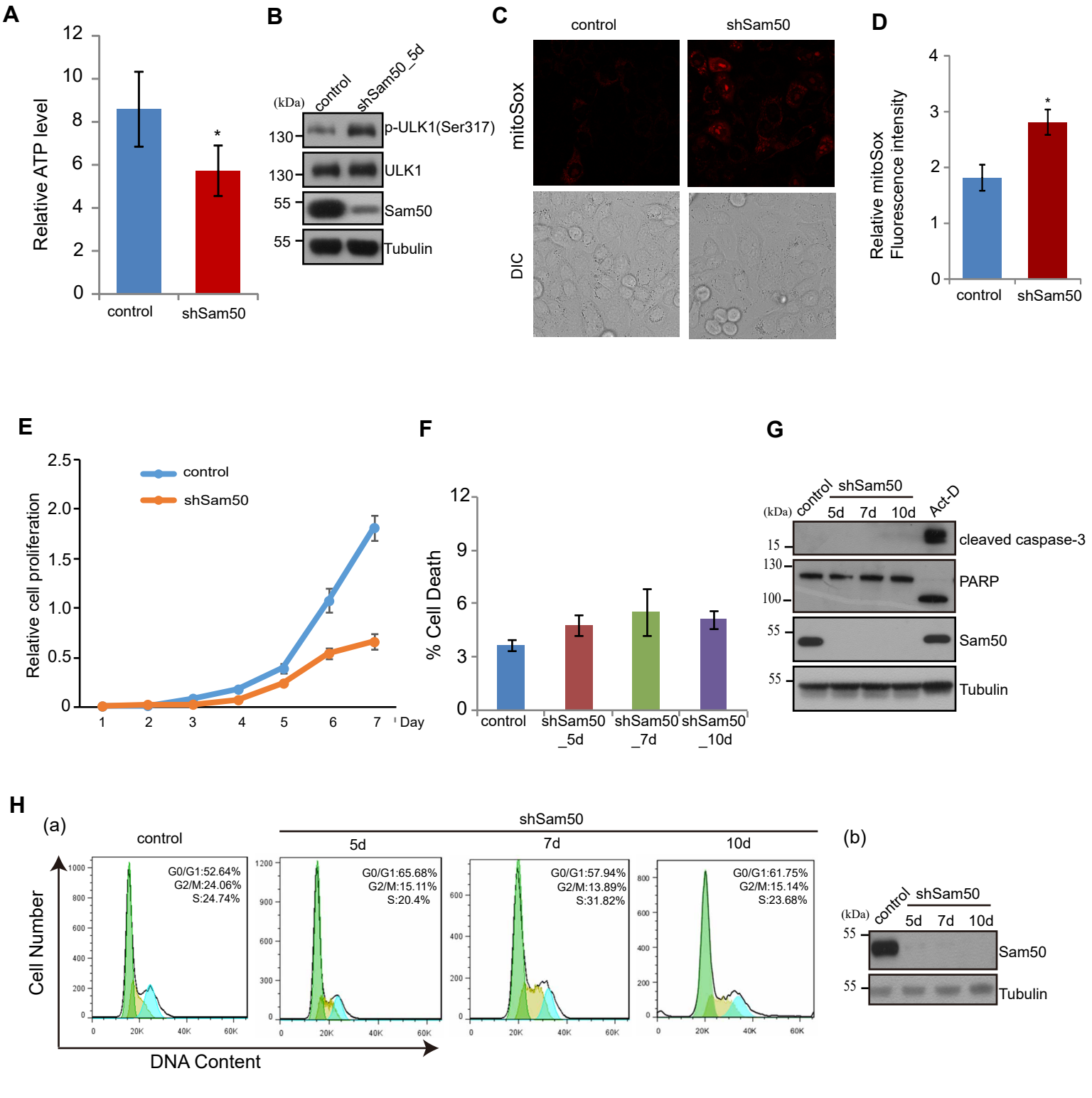
(E) Control or shSam50 (5 days) HeLa cells were immunoblotting using the indicated antibodies.

(F-H) the mitochondrial morphology of control, shSam50 (5 days), shSam50 (5 days) plus Flag-Mfn1, or shsam50 (5 days) plus Myc-Mfn2 HeLa cells was visualized by confocal microscope (F), then was counted according to the criteria detailed in "Experimental Procedures" (G). Data are expressed as means ± SD,*P<0.01. The protein levels of Mfn1, Mfn2 and Sam50 were analyzed by immunoblotting using the indicated antibodies (H).

(I) WT, Mfn DKO or OPA1 KO MEFs cells expressing mito-DsRed were infected with control or shSam50 lentiviral particles and cultured for 10 days, then the whole cell lysates were evaluated by immunoblot analysis using the indicated antibodies.

(J-K) control or shSam50 (5 days) HeLa cells lysates were used for co-immunoprecipitation assay by anti-Mfn2 antibody, and the eluted protein samples were analyzed by immunoblotting with anti-Mfn2 and anti-Mfn1 antibodies (J). The relative ratio of Mfn1/Mfn2 was quantified (K). Error bars indicate the mean ±SD of three independent experiments, **P<0.001.

(L-M) Control or shSam50(5d) HeLa cells expressing Myc-Mfn2 lysates were used for co-immunoprecipitation assay by anti-Myc antibody, and the eluted protein samples were analyzed by immunoblotting with anti-Mfn2 antibody (L). The relative ratio of Mfn2/Myc-Mfn2 was quantified (M). Error bars indicate the mean ±SD of three independent experiments **P<0.001.


A
Relative ATP level
12
10
8
6
4
2
0
control
shSam50
*
B
(kDa)
control
shSam50_5d
130
p-ULK1(Ser317)
130
ULK1
55
Sam50
55
Tubulin
C
control
shSam50
mitoSox
DIC
D
Relative mitoSox Fluorescence intensity
4
3
2
1
0
control
shSam50
*
E
Relative cell proliferation
2.5
2.0
1.5
1.0
0.5
0
control
shSam50
1
2
3
4
5
6
7
Day
F
% Cell Death
12
9
6
3
0
control
shSam50 _5d
shSam50 _7d
shSam50 _10d
G
(kDa)
control
shSam50
5d
7d
10d
Act-D
15
cleaved caspase-3
130
100
PARP
55
Sam50
55
Tubulin
H
(a)
control
shSam50
5d
7d
10d
Cell Number
G0/G1:52.64%
G2/M:24.06%
S:24.74%
G0/G1:65.68%
G2/M:15.11%
S:20.4%
G0/G1:57.94%
G2/M:13.89%
S:31.82%
G0/G1:61.75%
G2/M:15.14%
S:23.68%
DNA Content
(b)
(kDa)
control
shSam50
5d
7d
10d
55
Sam50
55
Tubulin

Supplemental Data

Figure S3 (Related to Figure 3): The effect of Sam50 knockdown in mitochondrial ATP or ROS production.

(A) Relative ATP levels of control or shSam50 HeLa cells were measured using an ATP assay kit. Bars represent means ± SD of three independent experiments.

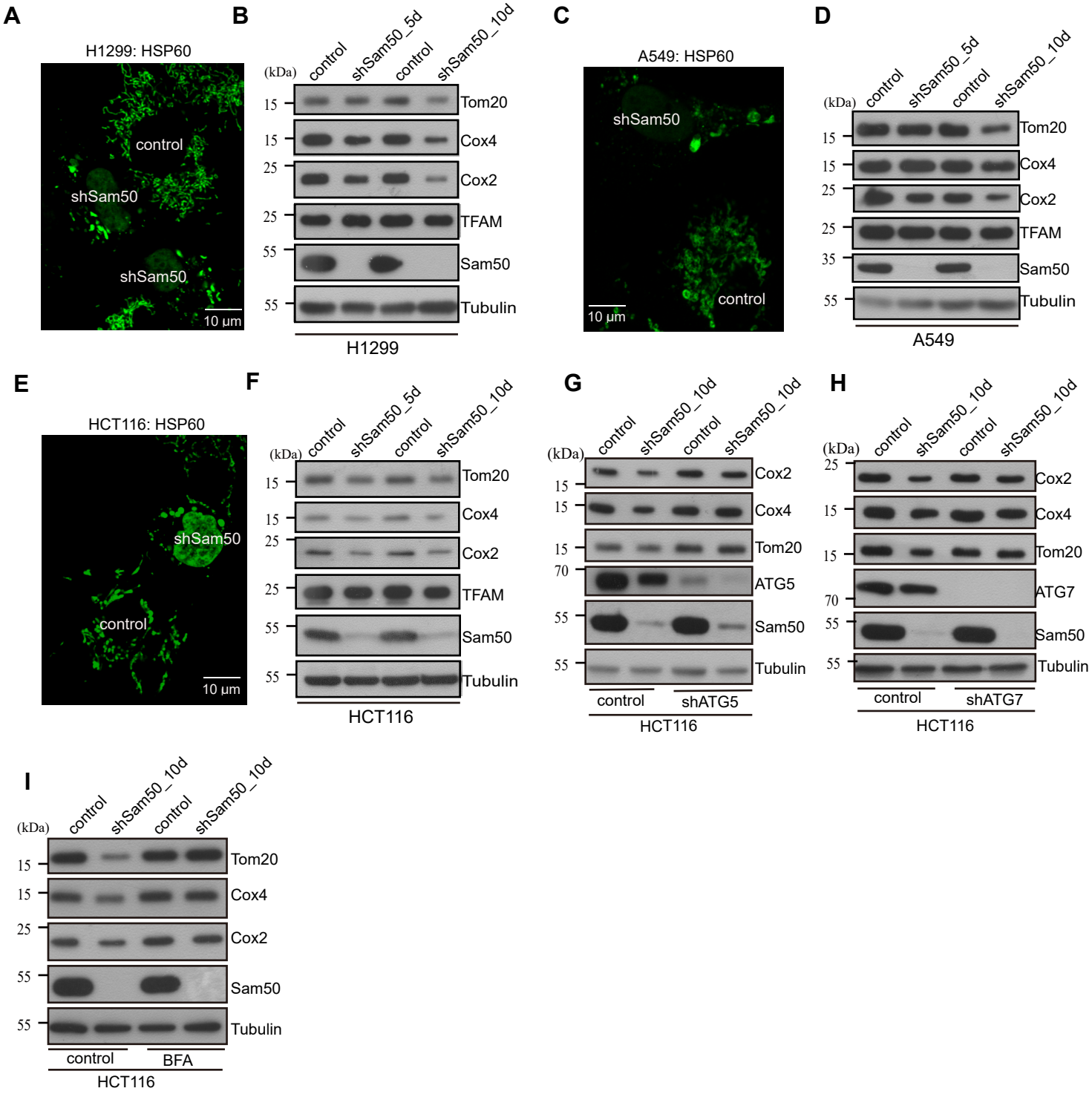
(B) Cell lysates of control or shSam50 (5 days) HeLa cells were measured by immunoblot analysis using the indicated antibodies.

(C and D) Control or shSam50 were HeLa cells were stained with MitoSox, cells were then visualized by confocal microscopy (C). The fluorescence intensity was further analyzed by ImageJ software (D). Bars represent means± SD of three independent experiments.

(E) The relative cell growth rate of control or shSam50 HeLa cells were measured by a cell counting kit (CCK-8). Bars represent means ± SD of three independent experiments.

(F-G) The percentage of cell death of control or shSam50 (5, 7, and 10 days) HeLa cells were quantified (F), bars represent means ± SD of three independent experiments. PARP and cleaved caspase-3 in cells described in "F" were measured by immunoblot analysis (G), and cells treated with actinomycin D (Act-D) acted as a positive control for apoptosis.

(H) Flow cytometry analysis of cell cycle in control or shSam50 (5, 7, and 10 days) HeLa cells (a). Immunoblot analysis of the protein level of Sam50 (b).


A
H1299: HSP60
control
shSam50
shSam50
10 μm
B
(kDa)
control
shSam50_5d
control
shSam50_10d
15
Tom20
15
Cox4
25
Cox2
25
TFAM
55
Sam50
55
Tubulin
H1299
C
A549: HSP60
shSam50
control
10 μm
D
(kDa)
control
shSam50_5d
control
shSam50_10d
15
Tom20
15
Cox4
25
Cox2
25
TFAM
35
Sam50
55
Tubulin
A549
E
HCT116: HSP60
shSam50
control
10 μm
F
(kDa)
control
shSam50_5d
control
shSam50_10d
15
Tom20
15
Cox4
25
Cox2
25
TFAM
55
Sam50
55
Tubulin
HCT116
G
(kDa)
control
shSam50_10d
control
shSam50_10d
15
Cox2
15
Cox4
15
Tom20
70
ATG5
55
Sam50
55
Tubulin
control
shATG5
HCT116
H
(kDa)
control
shSam50_10d
control
shSam50_10d
25
Cox2
15
Cox4
15
Tom20
70
ATG7
55
Sam50
55
Tubulin
control
shATG7
HCT116
I
(kDa)
control
shSam50_10d
control
shSam50_10d
15
Tom20
15
Cox4
25
Cox2
55
Sam50
55
Tubulin
control
BFA
HCT116

Supplemental Data

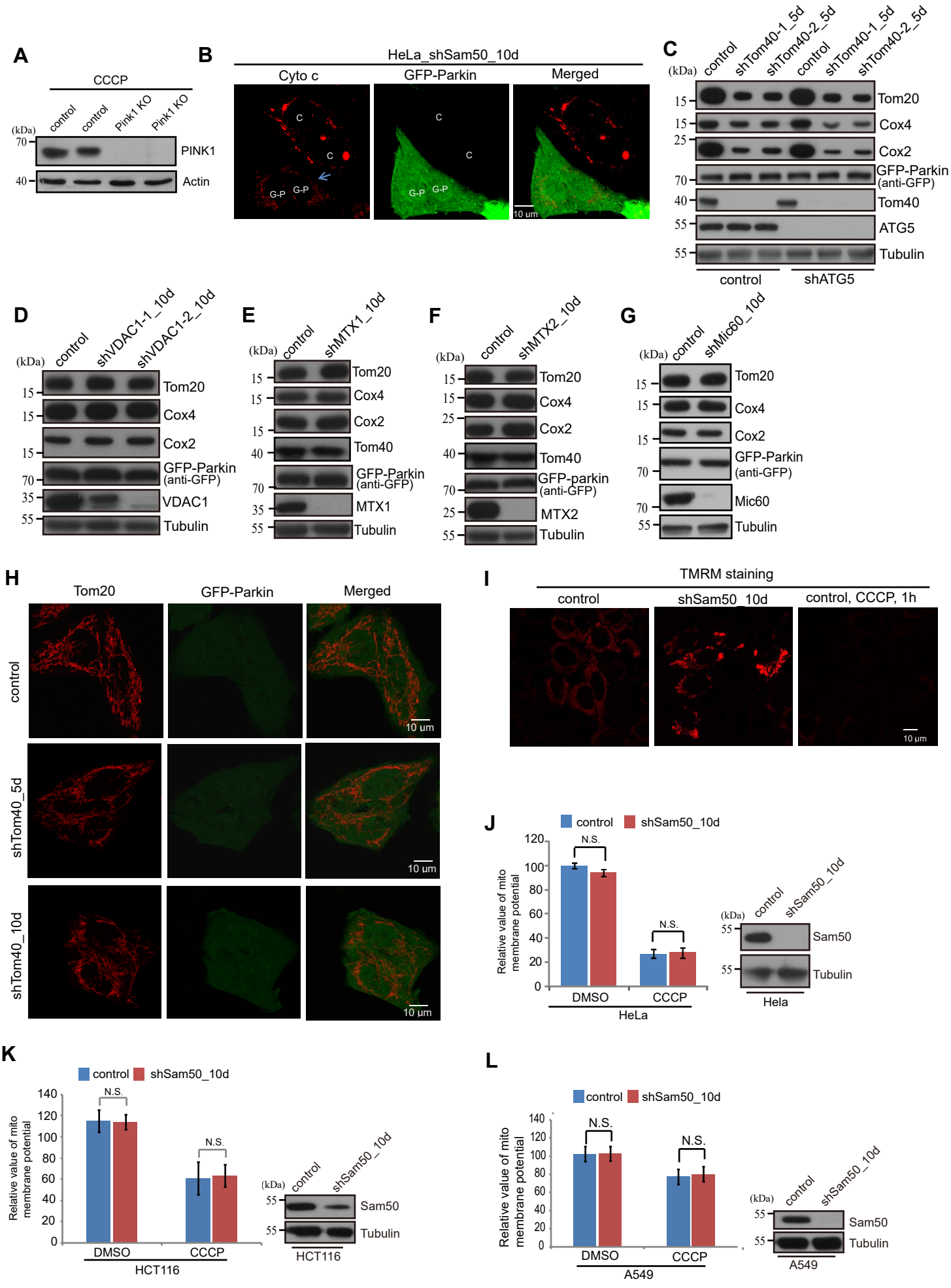
Figure S4 (Related to Figures 3 and 4): Sam50 knockdown induces mitophagy in some other cell lines.

(A-F) H1299 (A and B), A549 (C and D), and HCT116 (E and F) cells were infected with control or shSam50 lentiviral particles, cells were immunostained for HSP60. Mitochondrial morphology was visualized by confocal microscope. Cell lysates were subjected to immunoblot using the indicated antibodies. Nuclear-targeted GFP contained cells indicates shSam50 cells.

(G) Control or shATG5 HCT116 cells were infected with control or shSam50 lentiviral particles and then cultured for 10 days, cells lysates were then assessed by immunoblot using the indicated antibodies.

(H) HCT116 cells with or without a shATG7 background were infected with control or shSam50 lentiviral particles and then cultured for 10 days, cell lysates were evaluated by immunoblot analysis as indicated.

(I) control or shSam50 HCT116 cells were cultured for 6 days, then with or without 200nM BFA treatment for 72h. Cell lysates were evaluated by immunoblot analysis as indicated.


A
CCCP
control
control
Pink1 KO
Pink1 KO
(kDa)
70
40
PINK1
Actin
B
HeLa_shSam50_10d
Cyto c
GFP-Parkin
Merged
C
G-P
10 μm
C
control
shTom40-1_5d
shTom40-2_5d
control
shTom40-1_5d
shTom40-2_5d
(kDa)
15
15
25
70
40
55
55
Tom20
Cox4
Cox2
GFP-Parkin (anti-GFP)
Tom40
ATG5
Tubulin
control
shATG5
D
control
shVDAC1-1_10d
shVDAC1-2_10d
(kDa)
15
15
15
70
35
55
Tom20
Cox4
Cox2
GFP-Parkin (anti-GFP)
VDAC1
Tubulin
E
control
shMTX1_10d
(kDa)
15
15
15
40
70
35
55
Tom20
Cox4
Cox2
Tom40
GFP-Parkin (anti-GFP)
MTX1
Tubulin
F
control
shMTX2_10d
(kDa)
15
15
25
40
70
25
55
Tom20
Cox4
Cox2
Tom40
GFP-parkin (anti-GFP)
MTX2
Tubulin
G
control
shMic60_10d
(kDa)
15
15
15
70
70
55
Tom20
Cox4
Cox2
GFP-Parkin (anti-GFP)
Mic60
Tubulin
H
Tom20
GFP-Parkin
Merged
control
shTom40_5d
shTom40_10d
10 μm
I
TMRM staining
control
shSam50_10d
control, CCCP, 1h
10 μm
J
control
shSam50_10d
Relative value of mito membrane potential
120
100
80
60
40
20
0
N.S.
N.S.
DMSO
CCCP
HeLa
(kDa)
control
shSam50_10d
55
55
Sam50
Tubulin
Hela
K
control
shSam50_10d
Relative value of mito membrane potential
140
120
100
80
60
40
20
0
N.S.
N.S.
DMSO
CCCP
HCT116
(kDa)
control
shSam50_10d
55
55
Sam50
Tubulin
HCT116
L
control
shSam50_10d
Relative value of mito membrane potential
140
120
100
80
60
40
20
0
N.S.
N.S.
DMSO
CCCP
A549
(kDa)
control
shSam50_10d
55
55
Sam50
Tubulin
A549

Supplemental Data

Figure S5 (Related to Figures 4 and 6): The effect of Sam50 knockdown on mitochondrial membrane potential.

(A) Control or PINK1 KO HeLa cells were treated with CCCP (10 μM, 2h), and then subjected to immunoblot using anti-PINK1 or anti-Actin antibody.

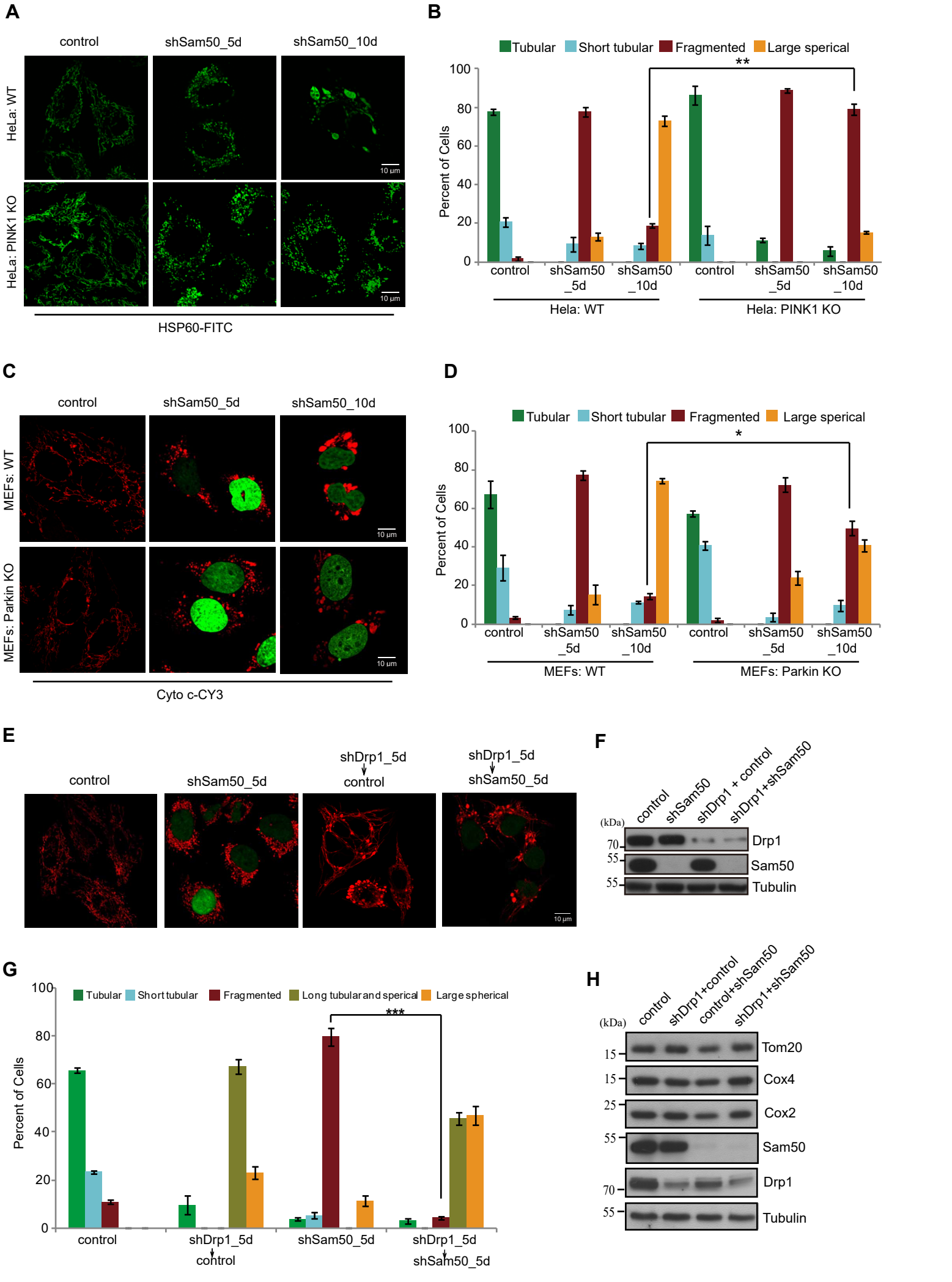
(B) Sam50 in control or GFP-Parkin expressed HeLa cells were knocked down for 10 days, the mitochondrial mass was evaluated by immunostaining for cytochrome c (Cyto c). "C" indicates "control cells", and "G-P" indicate "GFP-Parkin expressed cells".

(C-G) HeLa cells expressing GFP-parkin were infected with control, shTom40 (C), or shTom40 plus shATG5 (C), shVDAC1 (D), shMetaxin1 (E), shMetaxin2 (F), shMic60 (G) lentiviral particles and then cultured for 5 or 10 days, cell lysates were subjected to immunoblot using the indicated antibodies.

(H) HeLa cells stably expressing GFP-Parkin were infected with control or shTom40 lentiviral particles for 5 or 10 days (most cells were died in 10 days), and then were immunostained with anti-Tom20 antibody. Localization of GFP-Parkin was visualized by confocal microscope.

(I) Control or shSam50 HeLa cells with or without CCCP (10 μM, 1h) treatment were incubated with a mitochondrial membrane potential-dependent dye, TMRM (tetramethylrhodamine methyl ester, 600 nM, 25 min), and washed extensively. Live fluorescent images were obtained by confocal microscope.

(J-L) HeLa cells (J), HCT116 cells (K) or A549 cells (L) were infected with control or shSam50 lentiviral particles, and cultured for 10 days, then treated with or without CCCP (10 μM, 4h) and incubated with TMRM (600 nM, 25 min), then analyzed by flow cytometry. Bars represent means± SD of three independent experiments. "N.S." represent "not significant".


A
control
shSam50_5d
shSam50_10d
HeLa: WT
HeLa: PINK1 KO
10 μm
HSP60-FITC
B
Tubular
Short tubular
Fragmented
Large sperical
**
Percent of Cells
100
80
60
40
20
0
control
shSam50 _5d
shSam50 _10d
control
shSam50 _5d
shSam50 _10d
Hela: WT
Hela: PINK1 KO
C
control
shSam50_5d
shSam50_10d
MEFs: WT
MEFs: Parkin KO
10 μm
Cyto c-CY3
D
Tubular
Short tubular
Fragmented
Large sperical
*
Percent of Cells
100
80
60
40
20
0
control
shSam50 _5d
shSam50 _10d
control
shSam50 _5d
shSam50 _10d
MEFs: WT
MEFs: Parkin KO
E
control
shSam50_5d
shDrp1_5d
control
shDrp1_5d
shSam50_5d
10 μm
F
control
shSam50
shDrp1 + control
shDrp1+shSam50
(kDa)
70
55
55
Drp1
Sam50
Tubulin
G
Tubular
Short tubular
Fragmented
Long tubular and sperical
Large spherical
***
Percent of Cells
100
80
60
40
20
0
control
shDrp1_5d
control
shSam50_5d
shDrp1_5d
shSam50_5d
H
control
shDrp1+control
control+shSam50
shDrp1+shSam50
(kDa)
15
15
25
55
70
55
Tom20
Cox4
Cox2
Sam50
Drp1
Tubulin

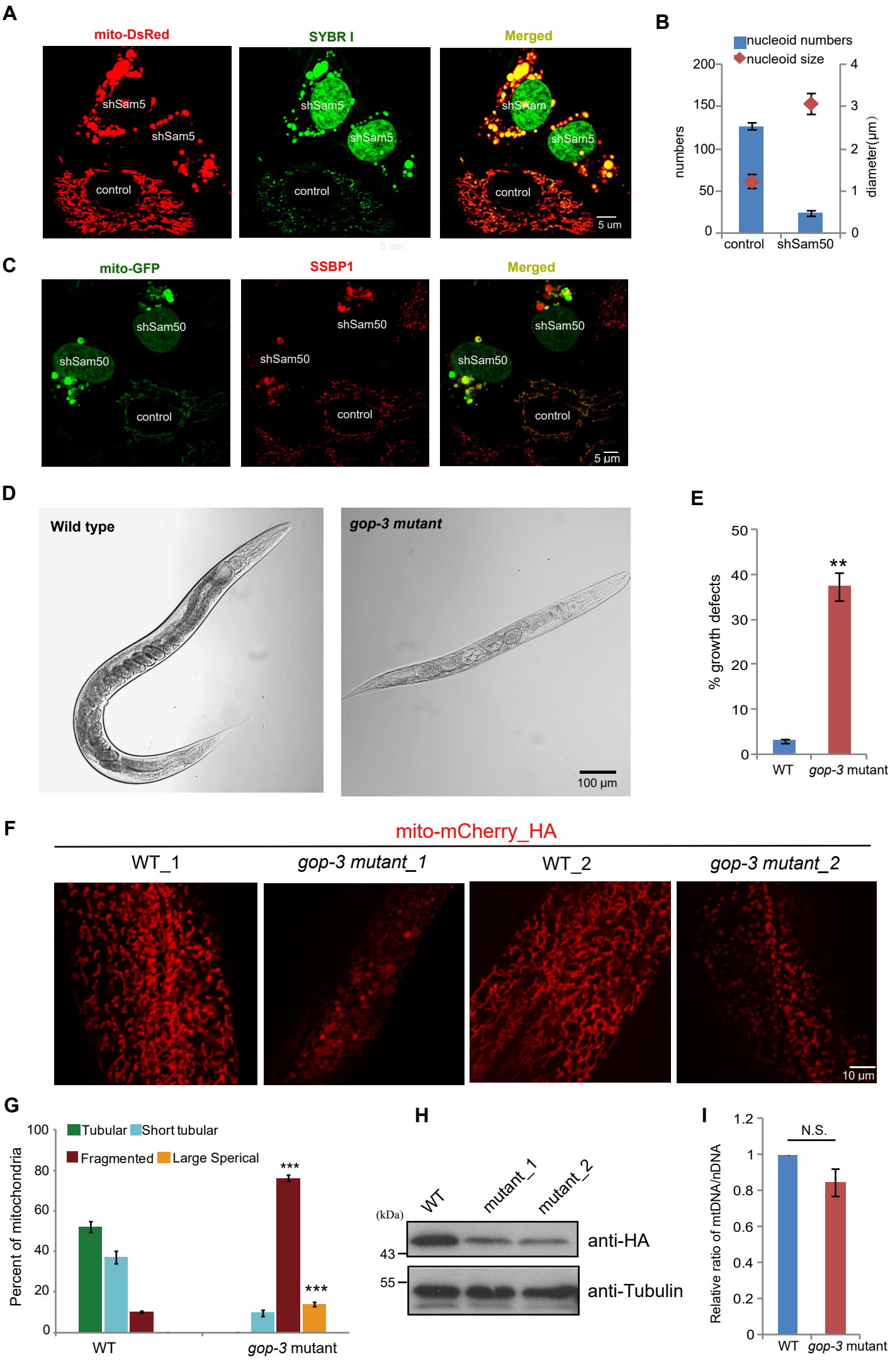
Supplemental Data

Figure S6 (Related to Figure 4): shSam50-induced mitochondrial fragmentation is independent on PINK1-Parkin.

(A-D) PINK1 knockout (KO) HeLa cells or Parkin knockout (KO) MEFs were infected with control or shSam50 lentiviral particles for 5 or 10 days, cells were immunostained with the indicated antibodies. Mitochondrial morphology was visualized by confocal microscopy (A and C), and was counted according to the criteria detailed in "Experimental Procedures". Bars represent means ± SD of triplicate counts of 100 cells (B and D).

(E-G) Control or shDrp1 HeLa cells expressing mito-DsRed were further infected with shSam50 lentiviral particles for additional 5 days. The mitochondrial morphology was visualized by confocal microscopy (E), and were counted according to the criteria detailed in "Experimental Procedures" (G), data are expressed as means ± SD, *$P<0.01$. The levels of Drp1 and Sam50 was analyzed by immunoblotting (F).

(H) Control or shDrp1 HeLa cells were further infected with shSam50 lentiviral particles for additional 10 days. Whole-cell lysates were analyzed by immunoblotting using the indicated antibodies.


A
mito-DsRed
SYBR I
Merged
shSam5
shSam5
control
shSam5
shSam5
control
shSAam
shSam5
control
5 um
B
nucleoid numbers
nucleoid size
numbers
diameter(μm)
200
150
100
50
0
4
3
2
1
0
control
shSam50
C
mito-GFP
SSBP1
Merged
shSam50
shSam50
control
shSam50
shSam50
control
shSam50
shSam50
control
5 μm
D
Wild type
gop-3 mutant
100 μm
E
% growth defects
50
40
30
20
10
0
**
WT
gop-3 mutant
F
mito-mCherry_HA
WT_1
gop-3 mutant_1
WT_2
gop-3 mutant_2
10 μm
G
Tubular
Short tubular
Fragmented
Large Sperical
Percent of mitochondria
100
80
60
40
20
0
***
***
WT
gop-3 mutant
H
WT
mutant_1
mutant_2
(kDa)
43
55
anti-HA
anti-Tubulin
I
Relative ratio of mtDNA/nDNA
1.2
1
0.8
0.6
0.4
0.2
0
N.S.
WT
gop-3 mutant

Supplemental Data

Figure S7 (Related to Figures 1 and 7): The role of Sam50 in the distribution of mtDNA nucleoids and the function of gop3 in *C. elegans*.

(A-B) Control or shSam50 HeLa cells expressing mito-DsRed were stained with SYBR green I. The morphology of mtDNA nucleoids was visualized by confocal microscope (A), cells containing nuclear-targeted GFP indicates shSam50 cells. The average number of mtDNA nucleoid per cell and average diameter of nucleoid were analyzed and quantified (B). 30 randomly selected cells were counted and analyzed. The data represent the means ± SD of three independent experiments.

(C) Control or shSam50 HeLa cells stably expressing mito-GFP were immunostained with an antibody against SSBP1. Morphology of mtDNA nucleoids was analyzed by confocal microscope.

(D-E) Wild type (WT) and *gop-3* mutant *C. elegans* were observed by DIC microscope (D). The percentage of growth defects was quantified. n=3 (100 worms per independent experiment), Data are expressed as means ± SD, **P<0.001 (E).

(F-G) Mitochondrial morphology in WT or *gop-3* mutant *C. elegans* was visualized by confocal microscope (F). Mitochondrial morphology was quantified. The data represent the means ± SD of three independent experiments (100 mitochondria was randomly selected per worm), ***P<0.0001 (G).

(H) Lysates of WT and *gop-3* mutant *C. elegans* were subjected to immunoblot using the indicated antibodies.

(I) mtDNA contents of WT or *gop-3* mutant *C. elegans* were quantified and normalized to nDNA. The data represent the means ± SD of three independent experiments. “N.S.” indicates “not significant”.

ADDITIONAL MATERIALS AND METHODS

Cell culture, Antibodies and Reagents

HeLa, A549, H1299, HCT116, 143B, 143B rho^0 cells and MEFs were cultured in Dulbecco's modified Eagle's medium (Gibco) supplemented with 10% FBS (PAN, Germany)，1% Penicillin/Streptamycin (Gibco) and 1% L-glutamine at 37°C with 5%CO_2, 143B rho0 are incubated with medium adding uridine (50μg/ml, Sigma Aldrich). Antibodies were used in this study: anti-Cox4, anti-Mff, anti-Tom20, anti-p62, anti-Tom40, anti-Metaxin-2, anti-Prohibitin-2, anti-Twinkle, and anti-TFAM ,anti-Syntaxin17, anti-MPPβ, anti-Mid49， anti-Mid51, anti-GM130, anti-Mic60, anti-Lon, anti-CLPP, anti-Afg3L2 and anti-OMP25 were purchased from Proteintech; anti-Cox2, anti-Mfn1, anti-Mfn2, anti-VDAC1, anti-POLG, and anti-Sam50 were from Abcam; anti-ATG5, anti-Dynamin2 and anti-Mic19 were from ABclonal; anti-LAMP1, anti-PARP, anti-PGC-1α, anti-GFP, anti-cytochrome c, and anti-HSP60 were from Santa Cruz Biotechnology; anti-PINK1 was from Novus Biologicals; anti-PMP70 was from Life technologies; anti-Mic10 was from Origene; anti-Drp1 and anti-OPA1 were purchased from BD Biosciences; anti-LC3 and anti-Flag was from Sigma-Aldrich; mouse monoclonal anti-DNA was from Progen; anti-Phospho-ULK1(Ser317), anti-ULK1, anti-ATG7, anti-Phospho-DRP1(Ser637), anti-Phospho-DRP1(616), anti-EEA1 and anti-cleaved Caspase-3 were purchased from Cell Signaling. Reagents used in this paper were: Carbonyl cyanide 3-chlorophenylhydrazone (CCCP, Sigma-Aldrich), oligomycin and antimycin-A (Sigma-Aldrich); actinomycin D (Abcam); SYBR Green I was purchased from Molecular Probes. Lipofectamine 2000 and Opti-MEM I (Invitrogen, Carlsbad, CA, USA).

Plasmids and shRNA construction

shRNAi against Sam50 was performed using a modified retroviral vector with the H1 promoter to drive the expression of shRNAs (Chen et al., 2005). The target sequences of shRNAi oligonucleotides were listed in "Table S1". GFP-Parkin, PINK1-GFP, Flag-Sam50, Myc-Sam50，and GFP-LC3 cDNA were cloned into pMSCV-puro or pHAGE constructs.

Immunoblot and Co-immunoprecipitation Assay

Whole-cell lysates were prepared with SDS-PAGE sample buffer. Samples were boiled for 10min, and then separated by SDS–PAGE and electrophoretically transferred onto a nitrocellulose membrane. After blocking with 5% nonfat milk in TBST, membrane was incubated with the primary antibodies, followed by HRP-conjugated goat anti-rabbit or anti-mouse IgG. Co-immunopricipitaion was performed as we have described previously (Li et al., 2016), and then the samples were analyzed by immunoblot as above described.

Photoactivatible GFP Assay

Mitochondrial targeted DesRed (mito-DsRed) and photoactivatible GFP (PA-GFP) targeted to mitochondrial matrix (mito-PA-GFP) were stably expressed in cells and performed PA-GFP assay. Within a single cell, a small subset of mitochondria were photoactivated by excitation at 405nm, and then the mitochondrial fusion and fission events were tracked by time-lapse microscopy for about 20 min.

Measurement of ATP Production.

Cellular ATP levels were measured using an ATP assay kit (Celltiter-Glo Luminescent Cell Viability Assay, Promega) according to the manufacturer's instructions. Luminescence was measured using microplate reader and the values were normalized to the protein concentration.

Measurement of Mitochondrial Membrane Potential

Cells in 6-well plates were collected and washed with PBS, and then cells were incubated at 37°C under 5% CO_2 with 600nM TMRM in DMEM without FBS for 25 minutes. Cells washed three times and analyzed by FACS. Cells were treated with Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) as a positive control.

Mitochondrial ROS Measurement

Mitochondrial ROS was detected by MitoSOX^{TM} Red (Invitrogen) according to the manufacturer's instructions. Briefly, cells were incubated with 5uM MitoSOX with Hank's balanced salt solution at 37°C for 15min, then washed cells 3 times with HBSS solution with Ca^{2+} and Mg^{2+} (1.26mM $CaCl_2$, 0.5mM $MgCl_2$, 0.4mM $MgSO_4$, 5.33mM KCl, 0.44 KH_2PO_4, 4.16mM $NaHCO_3$, 138mM NaCl, 0.34mM Na_2HPO_4 and 5.56mM D-Glucose). The fluorescence was measured qualitatively by confocal microscopy under excitation at 510nm, and the emission was collected at 580nm. The fluorescence intensity was analyzed by using LAS AF lite software.

Cell Cycle Analysis

Cell cycle was analyzed by flow cytometry. Cells were collected and washed with PBS, then cells were fixed with 70% ethanol overnight at 4 °C. The fixed cells were centrifuged at 800 g at 25 °C for 5 min, the supernatant was removed, and the cells were washed with PBS. Then, cells were stained with 4 μl of 10 mg/ml propidium iodide (PI) and 10 μl of 1 mg/ml RNase in 400 μl PBS, followed by incubation in the dark for 30 min at 4 °C prior to measurement of the stained cells on a flow cytometer (Beckman, Indianapolis, CA, USA). The results were analyzed using FlowJo software (Tree Star, San Carlos, USA).

Cell Proliferation and Cell Death Assay

Cell proliferation was measured using a Cell Counting Kit (CCK-8) according to the manufacturer's instructions. The absorbance of the control and shSam50 cells was measured with microplate reader at 450nm. For the cell death assay, a 10μl aliquot of cell suspension was incubated with 10μl of 0.4% trypan blue solution for 5 minutes at room temperature. Viable and nonviable cells based on absence and presence of intracellular trypan blue dye, respectively. Cells were counted by the hemacytometer.

Mito-Keima Mitophagy Assay

HeLa cells expressing Flag-Parkin were infected with a lentivirus harboring the mito-keima vector (a gift from Michael Lenard), and grown for several days. control, shSam50 or OA treated cells stably expressing Flag-Parkin plus mito-Keima were trypsinized, washed once with PBS buffer and then resuspended in sorting buffer (145mM NaCl, 5mM KCl, 1.8mM CaCl2, 0.8mM MgCl2, 10mM HEPES,10mM Glucose, 0.1% BSA), and analyzed by a BD flow cell sorter. Measurements of lysosomal mito-Keima were made using dual-excitation ratiometric pH measurements at 488 (pH 7) and 561 (pH 4) nm lasers with 620/29 nm and 614/20 nm emission filters, respectively. For each sample, 20,000 events were collected. Data were analyzed using FlowJo (Tree Star, San Carlos, USA).

EM Studies

HeLa cells were fixed and prepared as we have described previously (Li et al., 2016). The stained sections were imaged onto negatives using a Jeol electron microscope (Joel Ltd, Tokyo, Japan) operated at 80 kV.

Mitochondrial DNA content quantification.

DNA from HeLa cells was extracted using the gDNA Kit (BioMIGA) according to the manufacturer's instructions. The LightCycler 480 SYBR Green I master (RoChe) was used for quantitative PCR with an ABI PRISM 7000 Sequence Detector System (Applied Biosystems). The primer sets for amplification of mtDNA and β-globin nuclear DNA fragments were used as described previously (Miller et al., 2003). mtDNA contents were calculated by using nuclear DNA content as a reference.

Plasmids and *C. elegans* CRISPR/Cas9 strains heat-shock treatment

All strains were raised at 20°C on nematode growth medium (NGM) plates seeded with the *Escherichia coli* strain OP50. Three CRISPR/Cas9 and sgRNA plasmids (50 ng/μl) were injected into hermaphrodites using the selection marker Podr-1::dsRed to generate the triple conditional knockouts. 50 transgenic adult worms were placed on a

seeded NGM plate to lay eggs for 4 hours, and removed adult worms. After that, the eggs were heat-shocked at 31°C for 1 hour and then cultured at 20°C until the adult stage.

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SUPPLEMENTAL EXPERIMENTAL TABLE

Table S1 (Related to Figures 1-7). List of primers for construction of shRNAi plasmids.

Target gene	Oligonucleotide sequence
Homo Sam50 (shSam50-A)	F 5′-GATCCCCGGTCATCGATTCTCGGAATTTCAAGAGAATTCCGAGAATCGA TGACCTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGGTCATCGATTCTCGGAATTCTCTTGAAATTCCGAGA ATCGATGACCGGG-3′
Homo Sam50 (shSam50-B)	F 5′-CCGGGGACATTCACTGAAATCATCTCTCGAGAGATGATTTCAGTGAATGTCC TTTTTG-3′ R 5′-AATTCAAAAAGGACATTCACTGAAATCATCTCTCGAGAGATGATTTCAG TGAATGTCC-3′
Mus Sam50 (shSam50)	F 5′-GATCCCCGAGGAGATGTGAGCTTCATTTCAAGAGAATGAAGCTCACATC TCCTCTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGAGGAGATGTGAGCTTCATTCTCTTGAAATGAAGC TCACATCTCCTCGGG-3′
Homo Metaxin-1 (shMTX1)	F5′-CCGGAAGTGGTATGCAGAGGCTATGCTCGAGCATAGCCTCTGCATACCAC TTTTTTTG-3′ R5′-AATTCAAAAAAAGTGGTATGCAGAGGCTATGCTCGAGCATAGCCTCTGCATA CCACTT-3′
Homo Metaxin-2 (shMTX2)	F 5′-CCGGGGGAAGTCAAACGTAAGATGACTCGAGTCATCTTACGTTTGACTT CCCTTTTTG-3 R 5′-AATTCAAAAAGGGAAGTCAAACGTAAGATGACTCGAGTCATCTTACGTT TGACTTCCC-3
Homo VDAC1(shVDAC 1-1)	F 5′-CCGGAAAGTGACGGGCAGTCTGGAACTCGAGTTCCAGACTGCCCGTCAC TTTTTTTTG-3′ R 5′-AATTCAAAAAAAAGTGACGGGCAGTCTGGAACTCGAGTTCCAGACTGCC CGTCACTTT-3′
Homo VDAC1 (shVDAC1-2)	F 5′-CCGGCTCCAGGTTAAAGTTGATTCACTCGAGTGAATCAACTTTAACCTG GAGTTTTTG-3′ R 5′-AATTCAAAAACTCCAGGTTAAAGTTGATTCACTCGAGTGAATCAACTTT AACCTGGAG-3′
Homo Tom40 (shTom40-1)	F 5′-CCGGCAACTGGTTGGCAACGGTATTCTCGAGAATACCGTTGCCAACCAG TTGTTTTTG-3′ R 5′-AATTCAAAAACAACTGGTTGGCAACGGTATTCTCGAGAATACCGTTGCC AACCAGTTG-3′
Homo Tom40 (shTom40-2)	F 5′-CCGGGGTTGGCAACGGTAACGTTGGCTCGAGCCAACGTTACCGTTGCCA ACCTTTTTG-3′ R 5′-AATTCAAAAAGGTTGGCAACGGTAACGTTGGCTCGAGCCAACGTTACCG TTGCCAACC-3′

Homo ATG5 (shATG5)	F 5′-CCGGCCTTTCATTCAGAAGCTGTTTCTCGAGAAACAGCTTCTGAATGAA AGGTTTTTG-3′ R 5′-AATTCAAAAACCTTTCATTCAGAAGCTGTTTCTCGAGAAACAGCTTCTGA ATGAAAGG-3′
Homo OPA1 (shOPA1)	F 5′-GATCCCCGTTATCAGTCTGAGCCAGGTTCAAGAGACCTGGCTCAGACTG ATAACTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGTTATCAGTCTGAGCCAGGTCTCTTGAACCTGGCTC AGACTGATAACGGG-3′
Homo Drp1 (shDrp1)	F 5′-GATCCCCGAGGTTATTGAACGACTCATTCAAGAGATGAGTCGTTCAATA ACCTCTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGAGGTTATTGAACGACTCATCTCTTGAATGAGTCGT TCAATAACCTCGGG-3′
Homo Mfn1 (shMfn1)	F 5′-GATCCCCGATACTAGCTACTGTGAAATTCAAGAGATTTCACAGTAGCTA GTATCTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGATACTAGCTACTGTGAAATCTCTTGAATTTCACAG TAGCTAGTATCGGG-3′
Homo Mfn2 (shMfn2)	F 5′-GATCCCCGGAAGAGCACCGTGATCAATTCAAGAGATTGATCACGGTGCT CTTCCTTTTTGGAAA-3′ R 5′-AGCTTTTCCAAAAAGGAAGAGCACCGTGATCAATCTCTTGAATTGATCA CGGTGCTCTTCCGGG-3′
Homo Syntaxin17 (shSyntaxin17-1)	F5′-CCGGGGTAGTTCTCAGAGTTTGATTCTCGAGAATCAAACTCTGAGAACT ACCTTTTTG-3′ F5′-AATTCAAAAAGGTAGTTCTCAGAGTTTGATTCTCGAGAATCAAACTCTG AGAACTACC-3′
Homo Syntaxin17 (shSyntaxin17-2)	F5′-CCGGCGATCCAATATCCGAGAAATTCTCGAGAATTTCTCGGATATTGGAT CGTTTTTG-3′ R5′-AATTCAAAAACGATCCAATATCCGAGAAATTCTCGAGAATTTCTCGGATAT TGGATCG-3′
Homo Mic60 (shMic60)	F5′-GATCCCCGCCCGAATGACTCTAGAAATTCAAGAGATTTCTAGGGTCATT CGGGCTTTTTGGAAA-3′ F5′-AGCTTTTCCAAAAAGCCCGAATGACCCTAGAAATCTCTTGAATTTCTAGG GTCATTCGGGCGGG